

### REMARKS

By the present amendment, the Applicants amended the specification to include reference to the sequence ID numbers. In addition, the Applicants cancelled claims 1-571. Claims 572-597 are now pending in this application.

As requested by the Notice, this supplemental response includes a paper copy and 3.5" diskette containing the Sequence Listing for this case. Pursuant to 37 CFR 1.821-825, the Applicants submit that the information contained in the paper and electronic copies of the Sequence Listing are the same. No new matter has been introduced into this application as a result of the present amendment.

Finally, an executed Inventors Declaration shall be submitted shortly.

Prompt consideration and entry of this supplemental response is respectfully requested.

Respectfully submitted,



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## APPENDIX A

On page 189, line 13 to page 190, line 8, please amend the paragraph as shown below:

Nanoparticle-nucleic acid conjugates are prepared as described in Example 3. The oligonucleotide modifier used to prepare these conjugates have the following sequence: 5'SH(CH<sub>2</sub>)<sub>6</sub>-A<sub>10</sub>-CGCATTTCAGGAT 3' (2) (SEQ ID NO:74). As shown in Figure 56, biotin labeled oligonucleotide (1) [3' biotin-TEG-A<sub>10</sub>-ATGCTCAACTCT 5'] (SEQ ID NO:73) is prepared by literature procedure using a Biotin TEG CPG support (Glen Research, Sterling, Virginia; Catalog no. 20-2955-01). To make the streptavidin/DNA conjugate, streptavidin was reacted with 1 equivalent of biotin-modified oligonucleotides in 20 mM Tris (pH 7.2), 0.2 mM EDTA buffer solution at room temperature for 2 h on a shaker. Streptavidin complexed to different numbers of oligonucleotides were separated by ion exchange HPLC with 20 mM Tris (pH 7.2) and a 0.5 %/min gradient of 20 mM Tris, 1 M NaCl at a flow rate of 1 mL/min, while monitoring the UV signal of DNA at 260 nm and 280 nm. The streptavidin/biotin-modified oligonucleotide mixture showed four peaks at 45 min, 56 min, 67 min and 71 min, which correspond to the 1:1, 2:1, 3:1, and 4:1 oligonucleotide-streptavidin complexes, respectively. The 1:1 complex of streptavidin/oligonucleotide (10 uM, 5uL) was isolated and premixed with linker DNA [5'TACGAGTTGAGAATCCTGATTGCG3'] (3) (SEQ ID NO:76) (10uM, 5uL) in 0.3 M PBS (0.3 M NaCl, 10 mM phosphate buffer, pH 7) and then the mixture was added to the nanoparticle-oligonucleotide conjugates (10 nM, 130 uL) in 0.3 M PBS to prepare a nanoparticle-oligonucleotide-streptavidin conjugate. The solution containing nanoparticle-oligonucleotide conjugates, streptavidin/oligonucleotide, and linker DNA was frozen in dry ice for 10 min and thawed to facilitate DNA hybridization. After 12 hr, the solution was centrifuged at 10000 rpm for 20 min and supernatant was removed. Red oily precipitate of nanoparticle-oligonucleotide-streptavidin conjugate was redispersed in 0.3 M PBS buffer.

On page 192, line 2 to page 193, line 6, please amend the paragraph as shown below:

The methods for preparing oligonucleotide-modified 13 nm Au particles (2-Au) and linker DNA (3) were reported elsewhere. The oligonucleotide modifier used to prepare these conjugates have the following sequence: 5'SH(CH<sub>2</sub>)<sub>6</sub>-A<sub>10</sub>-

CGCATTTCAGGAT 3' (SEQ ID NO:75). The linker DNA (3) has the following sequence: [5'TACGAGTTGAGAATCCTGATTGCG3'] (SEQ ID NO:76). See J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **1998**, *120*, 1959. **2-Au** and **3** were stored in 0.3 M NaCl, 10 mM phosphate (pH 7) buffer (0.3 M PBS) prior to use. Biotin modified DNA (2) was synthesized with Biotin TEG CPG support (Glen Research) and purified by literature methods. See J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **1998**, *120*, 1959; T. Brown, D. J. S. Brown, in *Oligonucleotides and Analogues* (Ed.: F. Eckstein), Oxford University Press, New York, **1991**.] Streptavidin was purchased from Sigma and dissolved in 20 mM Tris buffer (30  $\mu$ M, pH 7.2). To make the streptavidin/DNA conjugate (**1-STV**), streptavidin was reacted with 8 equivalents of biotin-oligonucleotide conjugate **1** in 20 mM Tris (pH 7.2), 0.2 mM EDTA buffer solution at room temperature for 2 h on a shaker. The biotin-oligonucleotide conjugate has the sequence: [3' biotin-TEG-A<sub>10</sub>-ATGCTCAACTCT 5'] (SEQ ID NO:73). Mixtures with different ratios of streptavidin to DNA (1:0.4, 1:1, 1:4) were also prepared for HPLC analysis. The streptavidin/DNA conjugates were separated from excess DNA by ion exchange HPLC with 20 mM Tris (pH 7.2) and a 0.5 %/min gradient of 20 mM Tris, 1 M NaCl at a flow rate of 1 mL/min, while monitoring the UV signal of DNA at 260 nm and 280 nm. The 1:0.4 streptavidin/DNA mixture showed two peaks at 45 min and 56 min, and the 1:1 mixture showed four peaks at 45 min, 56 min, 67 min and 71 min, which correspond to the 1:1, 2:1, 3:1, and 4:1 oligonucleotide-streptavidin complexes, respectively. The 1:4 mixture showed four peaks at the same positions but with increased intensity of third (67 min) and fourth (71 min) peaks. The HPLC spectrum of 1:8 streptavidin/DNA mixture showed two main peaks, one at 59 min for unreacted DNA and the other at 71 min for the 4:1 oligonucleotide-streptavidin complex. In addition, a shoulder at 67 min assigned to 3:1 complex was also present. Purified streptavidin-biotinylated DNA conjugates were concentrated and dispersed in 0.3 M PBS by ultrafiltration (centricon 30). Aggregates for TEM, thermal denaturation experiments, and SAXS measurements were prepared by freezing the solution containing **1-STV**, **2-Au**, and **3** in dry ice for 10 min, and thawed prior to the measurement to facilitate hybridization.

On page 195, line 6 to page 196, line 8, please amend the paragraph has shown below:

An Au nanoparticle/protein assembly also could be formed through streptavidin/biotin interactions as opposed to hybridization-induced assembly. For example, in a typical experiment, **1** (0.24 mM, 1.3  $\mu$ L), **3** (10  $\mu$ M, 32  $\mu$ L) and **2**-modified nanoparticles (Au nanoparticle concentration: 9.7 nM, 260  $\mu$ L) were mixed in 0.3 M PBS buffer to generate biotin-modified Au particles. The mixture was heated to 60 °C for 10 min and then cooled to room temperature to facilitate hybridization. After 24 h, the solution containing **1**, **3**, and **2**-modified Au nanoparticles was added to streptavidin (10  $\mu$ M, 4.2  $\mu$ L) in 0.3 M PBS and heated to 50 °C to form the nanoparticle aggregates. The color of the solution turned purple indicating aggregate formation, and the aggregates could be disassembled to **2**-modified Au particles, **1**-modified streptavidin, and **3** by raising the temperature of the solution above the  $T_m$  (65 °C). Small Angle X-ray Scattering (SAXS) [S.-J. Park, A. A. Lazarides, C. A. Mirkin, P. W. Brazis, C. R. Kannewurf, R. L. Letsinger, *Angew. Chem. Int. Ed.* **2000**, 39, 3845; A. Guinier, F. G., *Small Angle Scattering of X-rays*, Wiley, New York, **1955**; B. A. Korgel, D. Fitzmaurice, *Phys. Rev. B* **1999**, 59, 14191] data were collected for aggregates formed from the Au and streptavidin building blocks (**1**-STV, **2**-Au) and two different lengths of DNA linkers (24-mer (**3**) and 48-mer) and compared to those for aggregates based on Au-Au assembly and the same linking oligonucleotides (**2**-Au, **4**-Au, 24-mer linker (**3**), 48-mer linker), Figure 59. The 48-mer linker was a duplex consisting of :

5'TACGAGTTGAGACCGTTAAGACGAGGCAATCATGCAATCCTGAATGCG (SEQ ID NO:29) and 3'GGCAATTCTGCTCCGTTAGTACGT (SEQ ID NO:30) (The duplex region is underlined.) containing 12-mer sticky ends which are complementary to **1** and **2**. For design of the SAXS experiments,  $A_{10}$  spacers in **1**, **2**, and **4**, which were used in the above experiments to provide more accessibility for DNA hybridization [See L. M. Demers, C. A. Mirkin, R. C. Mucic, R. A. Reynolds, R. L. Letsinger, R. Elghanian, G. Viswanadham, *Anal. Chem.* **2000**, 72, 5535, were removed to create a more rigid system. The aggregates linked by DNA showed relatively well defined diffraction peaks, and the Au-STV aggregates exhibited diffraction peaks at smaller  $s$  values than the Au-Au aggregates formed from the same linker. This suggests a larger Au interparticle distance in the Au-STV system. Furthermore, the diffraction peaks shift to smaller  $s$  values when 48-mer DNA was used as a linker instead of 24-mer for both the Au-Au and Au-STV assemblies, Figure 59.